

B' Cont'd

--11. A pertussis toxoid prepared by the method of claim

10.--

R E M A R K S

The claims are 2-5 and 7-11.

A favorable reconsideration is courteously requested in view of the foregoing amendment and the following remarks.

Claim 1 has been rewritten as new claim 10 in order to more clearly set forth the two novel steps distinguishing this industrial process for preparing the toxoid.

It will be noted that the functional terminology objected to by the Examiner in her rejection under 35 USC 112, has been eliminated except for the description of the preliminary step, in which the term "removing" has been maintained. As pointed out hereinbelow, the critical steps are the flocculation step and the dispersion step. It is well understood that, in claiming a sequence of procedures, a preliminary step can be defined by functional language, especially where the critical novel steps are clearly defined by operative language.

Regarding the dialysis treatment, it is stated at page 4, line 10 that it may be interposed between the respective steps. Dialysis is also described in Example 1, page 7, lines 15 ff. A withdrawal of the rejection under 35 USC 112 is, therefore, courteously requested.

The claims to the invention, now more clearly defined in generic claim 10, stands rejected under 35 USC 103 as being allegedly obvious from the disclosure of Ayme et al. in combination with four secondary references.

As stated in page 1 of the specification as filed, the proposition that the infection by *Bordetella pertussis* lies in the exotoxin release from the said bacteria (M. Pittmann:

"Reviews of Infectious Diseases", 1, pg. 401-412, 1979) suggested the possibility of protection by means of a pertussis toxoid. However, there has been no report indicating the success of obtaining a pertussis toxoid.

The present invention provides a successful method of producing the pertussis toxoid by the particular sequence of steps as defined in new claim 10. Specifically, the present invention is essentially characterized by the combination of a step of flocculating pertussis exotoxin (in the pertussis exotoxin fluid resulting from the removal of endotoxin) by permitting formaldehyde to act upon the fluid in the substantial absence of basic amino acid, and the further step of dispersing the flocculent toxoid mass in the resulting suspension by ultrasonication.

There is thus obtained a toxoid low in toxicity with a very high immunizing potency. This offers a purified pertussis-diphtheria-tetanus trivalent vaccine of low toxicity and very high immunizing potencies.

In sharp contrast thereto, such effects cannot be achieved with pertussis toxoid fluid prepared by permitting formaldehyde to act upon pertussis exotoxin fluid in the substantial presence of basic amino acid, especially L-lysine, which process of course does not cause the flocculation of the pertussis exotoxin and therefore does not comprise the step of dispersing the flocculent toxoid mass by ultrasonication. This fact is clear from Table 2 in Example 1 of the specification, as discussed on lines 27-29 of page 7 and lines 19-26 of page 8.

The cited references in no way suggest the steps of the instant invention nor the excellent effects attained thereby.

The primary reference of Ayme et al. teaches only a process for separating lipid fractions from the bacterial endotoxin such as *Bordetella pertussis* endotoxin by hydrolyzing the latter and therefore is not applicable to the present invention in which *Bordetella pertussis* exotoxin is detoxified.

The secondary references relate to detoxification of bacterial exotoxin or a component thereof using formaldehyde. However, none of them teach either the step of flocculating pertussis exotoxin by permitting formaldehyde to act upon the exotoxin fluid in the absence of basic amino acid nor the step of dispersing the flocculent pertussis exotoxin mass by ultrasonication.

It is especially emphasized that the dispersion of the flocculent exotoxin mass by ultrasonication to give a toxoid fluid was entirely unobvious, especially because of the teaching in the prior art that flocculation of a toxoid or vaccine causes lowering of immunizing potency.

In view of the total novelty of the two essential steps featured in the process of claim 10, leading to unexpected and highly useful results, a favorable reconsideration of all the claims is courteously requested.

Respectfully submitted,
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